(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 February 2005 (17.02.2005)

PCT

(10) International Publication Number
WO 2005/014798 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number:
PCT/US2004/009829

(22) International Filing Date: 31 March 2004 (31.03.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/459,185 31 March 2003 (31.03.2003) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOVEL COMPOSITIONS AND METHODS FOR PROMOTING, INHIBITING, AND DETECTING PROTEIN ENTRY INTO CELLS

(57) Abstract: In vitro delivery of the diphtheria toxin (DT) catalytic (C) domain from the lumen of purified early endosomes to the external milieu requires the addition of both ATP and a cytosolic translocation factor (CTF) complex. The results presented here demonstrate that both Hsp 90 and TrR-1 activity plays an essential role in the cytosolic release of the C-domain and is mediated by a consensus peptide sequence found on several bacterial toxins and in HIV-1 reverse transcriptase. The invention features methods for inhibiting cell death that include the administration of compounds based on this consensus sequence that inhibit the translocation of the catalytic domain of toxins or transcription factors. Also featured are methods for identifying compounds that inhibit cell death, and methods for identifying compounds that promote cell death by blocking or accelerating, respectively, the rate of toxin/factor endosomal translocation.

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Toxin/Transcription Factor	Sequence No.	Sequence (single AA code)
Anthrax toxin-EF	(17-29)	E K N K T E K E K F K D
Anthrax toxin-EF	(405-415)	K L D H L R I E E L K E
Anthrax toxin-LF	(28-39)	E R N K T Q E E H L K E
Botulinum A	(720-731)	A K V N T Q I D L I R N
Botulinum A	(828-839)	T L I N G Q V D R L K D
Botulinum Cl	(755-766)	E N I K S Q V E N L K N
Botulinum D	(751-762)	E K I K S Q V E N L K N
Diphtheria toxin	(211-222)	D K T K T K I E S L K E
HIV-1 reverse transcriptase Patent No. 1		D K H R T K I E E L R Q
HIV-1 reverse transcriptase Patent No. 1		Q K N R T K I E E L R E
HIV-1 reverse transcriptase Patent No. 1		E K H R T K I E E L R E
HIV-1 reverse transcriptase Patent No. 1		G R H K T R I E E L R E
HIV-1 reverse transcriptase Patent No. 1		D K H R T K I E E L K E
HIV-1 reverse transcriptase Patent No. 1		Q G H K T K I E E L K E
CONSENSUS SEQUENCE		E K x K T x x E x L K E

Fig. 9

715-733

RESULT 3
ADY20753
ID ADY20753 standard; peptide; 12 AA.
XX
AC ADY20753;
XX
DT 05-MAY-2005 (first entry)
XX
DE Botulinum peptide fragment #1.
XX
KW Delivery mechanism; toxin; endocytosis; bacterial infection;
KW viral infection; antibacterial; virucide.
XX
OS Unidentified.
XX
PN WO2005014798-A2.
XX
PD 17-FEB-2005.
XX
PF 31-MAR-2004; 2004WO-US009829.
XX
PR 31-MAR-2003; 2003US-0459185P.
XX
PA (BOST-) BOSTON MEDICAL CENT CORP.
XX
PI Murphy JR, Ratts R, Pearson DA;
XX
DR WPI; 2005-173098/18.
XX
PT New compound, useful in the manufacture of a medicament for inhibiting
PT cell death or the translocation of a viral or bacterial toxin or viral
PT transcription factor for treating or preventing bacterial or viral
PT infections.
XX
PS Disclosure; Fig 9; 100pp; English.
XX
CC The invention relates to a new peptide compound and a nucleic acid
CC sequence encoding the peptide. The invention also relates to a method of
CC identifying a compound that inhibits cell death in a mammal and a method
CC of identifying a compound that promotes cell death in a mammal. The
CC compound is useful in the manufacture of a medicament for inhibiting cell
CC death in a mammal. The compound inhibits the translocation of a viral or
CC bacterial toxin from the lumen of an endosome to the cytosol of the cell
CC or the translocation of a viral or retroviral transcription factor. The
CC compound is further reacted with a monoclonal antibody, or its fragment
CC to form a covalent bond between a sulfur atom of the antibody and the
CC maleimide group of the compound. Identifying a compound that inhibits
CC cell death in a mammal comprises isolating endosomes from the cell,
CC placing the endosomes in a cytosolic buffer, contacting the endosomes
CC with a fusion protein-toxin, where the protein comprises a binding moiety
CC for a component of the cell membrane of the cell and the toxin comprises
CC a fragment of Diphtheria toxin, contacting the endosomes with a cytosolic
CC translocation factor complex, contacting the endosomes with the compound
CC and measuring translocation of the toxin, where a decreased level of the
CC translocation relative to that observed in the absence of the compound
CC indicates that the compound inhibits the cell death. Identifying a
CC compound that promotes cell death in a mammal comprises isolating
CC endosomes from the cell, placing the endosomes in a cytosolic buffer,
CC contacting the endosomes with a fusion protein-toxin, where the protein
CC comprises a binding moiety for a component of the cell membrane of the
CC cell and the toxin comprises a fragment of Diphtheria toxin, contacting

CC the endosomes with a cytosolic translocation factor complex, contacting
CC the endosomes with the compound and measuring translocation of the toxin,
CC where an increased level of the translocation relative to that observed
CC in the absence of the compound indicates that the compound promotes the
CC cell death. The compound is useful in the manufacture of a medicament for
CC inhibiting cell death in a mammal or for inhibiting the translocation of
CC a viral or bacterial toxin, e.g., Diphtheria toxin, a Botulinum toxin,
CC Anthrax toxin LF or Anthrax toxin EF from the lumen of an endosome to the
CC cytosol of the cell or the translocation of a viral or retroviral
CC transcription factor, e.g., human immunodeficiency virus reverse
CC transcriptase or Tat for treating or preventing bacterial or viral
CC infections. This sequence represents a botulinum peptide fragment used in
CC the scope of the invention.
XX
SQ Sequence 12 AA;

Query Match 53.1%; Score 52; DB 9; Length 12;
Best Local Similarity 100.0%; Pred. No. 0.13;
Matches 11; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

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Qy          5 AKVNTQIDLIR 15
              ||||||||||
Db          1 AKVNTQIDLIR 11
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